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(54) Title: PREBIOTICS AND PROBIOTICS

(57) Abstract

A method of enhancing a resident population of lactic acid-producing microorganisms, preferably lactobacillus, in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of the resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided.

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Prebiotics and probiotics

Technical Field

This invention relates generally to the use of prebiotic and probiotic preparations to improve intestinal health and conditions related to microbial populations in the gastrointestinal tract of host humans and animals, and thereby improve the health and well being of the host.

Background Art

The concept of probiotics was in use in the early 1900s, however, the term was only coined in 1965 and has subsequently evolved and numerous definitions have been proposed. Initially it was used to refer to the stimulation of the growth of one microbe by another i.e. the opposite of antibiotic. Today it is generally agreed that a probiotic is a preparation "of live microorganisms which, applied to human or animal, beneficially affects the host by improving the properties of the indigenous microbiota". Lactic acid bacteria and particularly lactobacilli are frequently used as probiotics. Bifidobacteria are also extensively used as probiotics. Furthermore, microbes of gastrointestinal origin or strains related to such microbes may also be used as probiotics.

The gastrointestinal (indigenous) microbiota contributes significantly to the health and well being of the host. It is a complex and diverse population which can reach up to 10^{14} bacteria in an individual and may have both beneficial and harmful effects on the host. Some of the beneficial and harmful effects of the gastrointestinal microbiota are summarised in Table 1. It is envisaged that these parameters may be influenced by probiotic administration.

The gut microflora of an healthy subject protects the host from pathogen invasion, however, in the young, the elderly, and the compromised patient, this protective barrier is less effective. An individual can be compromised to various degrees, varying from minor stress related events to extreme cases such as in immunocompromised patients and patients undergoing therapy.

The term prebiotic has been coined to refer to carbohydrate preparations that may be preferentially used to provide a carbohydrate source for desirable microbes.

TABLE 1
Influences of the human intestinal microbiota on the host

Beneficial effects	Harmful effects
Inhibition of pathogens	Constipation
Stimulation of immune system	Diarrhoea
Synthesis of vitamins	Infections
Aid in digestion	Liver damage
Produce metabolic fuel for enterocytes	Cancer
Maintain stability of ecosystem	Flatulence
Metabolise drugs	Irritable bowel
	Ulcerative colitis
	Crohn's disease

Glucans are cell wall polysaccharides with glucose as the main component sugar having a beta-bonding with mostly beta 1.3 links and also beta 1.6 links. While originally isolated from black fungi, glucans have since been found in large quantities in yeast cell walls. Glucans have been shown to propagate indigenous bifidus bacteria in intestines and have been proposed to be useful as a pharmaceutical agent for conditioning the intestine. It has been reported that glucans can function as an immunostimulator (e.g. Brattgjerd et al 1994) when intraperitoneally administered.

Probiotic bacteria have been described to exert antimicrobial effects which refers to the actions of the probiotic preparation on another microbe or group of microbes. Some probiotic bacteria are directly applicable for enhancing resistance against intestinal pathogens, prevention of diarrhoea and constipation (reviewed by Fernandes *et al.*; 1987; Katelaris, 1996; Sanders, 1994). The types of interactions include competitive colonisation as well as adhesion and growth inhibition.

Competitive colonisation refers to the fact that the probiotic strain can successfully compete with the pathogen for either nutrients or the site of colonisation. As many gastrointestinal pathogens attach to the intestinal mucosa as the first step in infection, it would be beneficial to the host if this adhesion could be inhibited. There are reports that lactobacilli produce

components which inhibit attachment of enterotoxigenic *Escherichia coli* to intestinal mucosa (Blomberg *et al.*, 1993), however, there is no evidence as yet that this occurs in the digestive tract. In addition, various compounds produced during growth of the probiotic have been shown to inhibit pathogen growth (reviewed by Fernandes *et al.*, 1987; Klaenhammer, 1988; Mishra and Lambert, 1996). These include organic acids such as lactic and acetic acid, reuterin and bacteriocins (Tagg *et al.*, 1976). The organic acids lower the pH and thereby can indirectly affect growth of the pathogen. In addition, the lactic and acetic acids produced by these organisms can be toxic to microbes. Reuterin which inhibits the growth of a very broad range of cells (Lindgren & Dobrogosz, 1990), is produced by *Lactobacillus reuteri* when grown in the presence of glycerol. Numerous bacteriocins have been reported to be produced by lactobacilli e.g. Acidophilin, Acidolin, Lactocidin, Bacteriocin, Bulgarican, Lactolin, Lactobacillin and Lactobrevin (reviewed by Fernandes *et al.*, 1987; Klaenhammer, 1988). Bacteriocins can either have a very broad range of activity or alternatively specifically inhibit the growth of a very limited range of closely related microbes. For example, *Lactobacillus* sp exhibited specific antagonistic effects towards *Clostridium ramosum* (McCormick & Savage, 1983).

The present inventors have surprisingly found that beta-glucans can be used to influence pathogen adhesion, alter microbial populations in the gastrointestinal tract, improve the effectiveness of probiotic compositions, and promote growth of a probiotic microorganism. A range of crude and pure preparations of beta-glucans have been shown to be effective.

Disclosure of the Invention

In a first aspect, the present invention consists in a method of enhancing a resident population of lactic acid-producing microorganisms in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of the

resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided.

In a second aspect, the present invention consists in a method of suppressing an undesired population of microorganisms in a total microorganism population in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of a resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided, and suppressing the growth and/or activity of the undesired population of microorganism

In a preferred form, the undesired population of microorganisms is enteric pathogens. As will be appreciated, there are a large number of pathogens including Gram negative bacteria like *Salmonella*, *Escherichia coli*, *Campylobacter*, *Helicobacter*, *Vibrio*, and *Pseudomonas*; Gram positive bacteria like *Clostridium*; viruses like Norwalk virus, Norwalk-like viruses and Rotavirus; and protozoa like *Cryptosporidium*, *Entamoeba*, *Giardia*, and *Dientamoeba*. The present invention would be suitable for the suppression of many of these pathogens in the gastrointestinal tract of animals.

Suitable beta-glucans include those originating from (a) plants including cereals such as oats and barley, (b) fungi, (c) yeast, and (d) bacteria. In addition, microbial cell wall preparations and whole cells rich in beta-glucans are also suitable sources for beta-glucan preparations useful for the present invention. Monomer residues in glucans can have 1-3 and 1-4, or 1-3 and 1-6 linkages (that is the monomer units are joined through 1,3, 1,4 or 1,6 bonds) and the percent of each type can vary. Preferably, beta-glucans derived from yeast, particularly from *Saccharomyces*, preferably *Saccharomyces cerevisiae*, are used for the present invention. It will be appreciated, however, that other beta-glucans would also be suitable. A concentration of up to about 10% when combined with other prebiotics

and/or probiotic microorganisms can be used. One percent has been found to be particularly suitable.

The resident lactic acid-producing microorganism is preferably a lactobacillus, and more preferably *Lactobacillus fermentum*.

5 In a preferred form, the present invention further includes one or more oligosaccharides, polysaccharides or other prebiotics. A concentration of oligosaccharides, polysaccharides or other prebiotics of about 0.001 g to 10 g per kg body weight per day is preferred.

10 Preferably, the probiotic microorganism is selected from the group consisting of lactic acid-producing microorganisms, bifidobacterium, yeast, and mixtures thereof. Preferably, the lactic acid-producing microorganism is a lactobacillus, more preferably *Lactobacillus fermentum*. Preferably, the yeast is a *Saccharomyces sp.*

15 Typical concentration range of probiotic microorganisms is 10^3 to 10^{13} cells per day.

20 Although oligosaccharides have been shown to promote bifidobacteria numbers in animals, prior to the present invention, it was thought that lactobacillus numbers cannot be changed in a similar manner. Gibson et al 1995 found that oligofructose and inulin, when fed to humans, selectively stimulated the growth of bifidobacteria without influencing the numbers of lactobacillus. From these results and observations by others in the field, it would not be expected that beta-glucans would positively effect the growth and/or activity of lactic acid-producing microorganisms, particularly lactobacillus, *in vivo* as found by the present inventors.

25 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

30 In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples and drawings.

Brief Description of Drawings

Figure 1 shows the results of *Lactobacillus* in the gastrointestinal tract of animals fed beta-glucan or control diet; and

5 Figure 2 shows faecal beta-glucanolytic microorganisms of animals fed beta-glucan or control diet. Analysis of beta-glucanolytic activity on agar plates containing beta-glucan from yeast cell sacs.

Modes for Carrying Out the Invention

EXAMPLE 1

This has been studied by enumerating coliforms, bifidobacterium and lactobacilli *in vitro* in the presence of beta-glucan. The beta-glucan used in 10 this example was prepared from yeast cell sacs. More specifically, aliquots (1 ml) of human faecal homogenates (10 g per 100 ml diluent) were added to diluted WC broth (diluted 50:50 with 0.05M phosphate buffer) to which were added the beta-glucan and a lactobacillus or bifidobacterium strain and various combinations. For each of the combinations, parallel tubes were 15 prepared with one set being inoculated with *Bifidobacterium* spp or *Lactobacillus* spp. All mixtures were then incubated for up to 24 hours and bacterial numbers enumerated. Results (Table 2) are expressed as the numbers of coliforms, total lactobacilli, total bifidobacterium, or total anaerobes when enumerated as colony forming units. It can be seen that 20 coliform numbers are unchanged or reduced, while numbers of bifidobacteria and lactobacilli can be increased up to 10-fold, and selected groups within a genus can be enhanced.

7

TABLE 2
Effect of 1% beta-glucan on *Lactobacillus* and *Bifidobacterium*

Culture mix	Coliforms	Bacterial numbers (CFU/ml)			<i>Bifidobacterium</i>	
		<i>Lactobacillus</i>		Large Colony		
		Total	Large Colony			
Faecal homogenate (FH)	3.2 x 10 ⁵	3.4 x 10 ⁵	1.2 x 10 ⁵		<2	
FH + 1% glucan	5.2 x 10 ⁶	5.3 x 10 ⁵	4.8 x 10 ⁵		<2	
FH + <i>Bifidobacterium</i> (SUB)	NA	NA	NA		1.0 x 10 ⁶	
FH + SUB + 1% glucan	NA	NA	NA		1.2 x 10 ⁷	
FH + <i>L. fermentum</i> (Lf)	NA	2.8 x 10 ⁵	2.8 x 10 ⁵		NA	
FH + Lf + 1% glucan	NA	1.2 x 10 ⁶	1.2 x 10 ⁶		NA	

NA - not analysed

5

EXAMPLE 2

This example demonstrates that dietary supplementation with beta-glucan stimulates gastrointestinal populations of *Lactobacillus*. In this study, animals (8 per group) were provided either beta-glucan diet or control diet

10 (Table 3). The beta-glucan used in this example was prepared from yeast cell sacs. After 6 weeks feeding, animals were euthanised and luminal content collected from stomach, ileum, caecum and colon. Collected material was homogenised and diluted in Wilkens-Charlgren broth (Oxoid). Dilutions were spread on to Rogosa gear (Oxoid) and incubated anaerobically for 48 h.

15 Beta-glucan-rich diet resulted in significant increase ($P < 0.0005$) of *Lactobacillus*, particularly in the gastric region where an 100 fold increase was detected (Fig. 1). The increase of lactobacilli numbers in the subsequent intestinal tract corresponded to about 1.5 log units.

TABLE 3
Composition of beta-glucan and sucrose diet

Ingredient	Amount (Glucan diet)	Amount (Control diet)
Beta-glucan	450	0
Sucrose	150	600
Casein	200	200
Sunflower	25	25
Canola oil	25	25
Gelatin	20	20
Wheat bran	100	100
Choline chloride	2	2
Methionine	3	3
Standard mineral mix	35	35
Standard vitamin mix	10	10

5 **EXAMPLE 3**

In addition to studying effects on microbial growth, effects on adhesion have been investigated. Desirable bacteria such as lactobacillus, as well as undesirable pathogenic *E. coli* and *Salmonella* sp have been grown either in a standard laboratory broth or in this broth supplemented with beta-glucans. The beta-glucan used in this example was prepared from yeast cell sacs. Bacterial suspensions were incubated with mucosal pieces from various regions of the gastrointestinal tract. These mucosal pieces were either used directly or after pre-treatment with the beta-glucan. Results are shown in Table 4. While adhesion of the pathogen was adversely affected, the adhesion of the lactobacillus was increased by the presence of the beta-glucan.

TABLE 4
Effect of beta-glucans on adhesion

Bacteria	Bacteria adhering (per mg tissue)		
	Control tissue	Tissue + 1% glucan	Reduction (%)
<i>S. typhimurium</i>	1.69×10^8	5.53×10^7	~ 65
<i>E. coli</i> K88	6.07×10^7	3.43×10^7	~ 50
<i>L. fermentum</i>	5.37×10^8	6.2×10^8	0

EXAMPLE 4

This example demonstrates that dietary supplementation with beta-glucan increases resistance to gastrointestinal infection by *Clostridium difficile* and *S. typhimurium* and that this effect is enhanced when glucan-treated mice were also orally dosed with *Saccharomyces sp* cell suspension.

The beta-glucan used in this example was acid and alkali washed cell sacs of yeast. The *Saccharomyces sp* alone reduced numbers of *C. difficile* to some extent and markedly reduced *S. typhimurium* numbers. Groups of animals (4 groups, 8 animals per group) were provided either beta-glucan diet or control diet (Table 3). After feeding diets for four weeks, four groups were supplied drinking water containing cefoxitin (0.032 g/L) and D-cycloserine (1.0 g/L) and subsequently challenged with *C. difficile* (10^{10} CFU). Another four groups were supplied drinking water containing streptomycin (5 g/L) and neomycin (5 g/L). These animals were subsequently challenged with *S. typhimurium* (10^7 CFU). After pathogen challenge, animals were orogastrically dosed with either *Saccharomyces sp* (strain FII 542900) (10^3 CFU in YPG broth), or with fresh YPG broth. Development of infections were monitored daily by analysis of faecal *S. typhimurium* or *C. difficile*. Faecal material was homogenised and diluted in Wilkins-Charlgen broth (Oxoid). Dilutions were spread on to either YSA (Oxoid) or *C. difficile* selective agar (Oxoid) for analysis of faecal *S. typhimurium* and or *C. difficile*, respectively.

Animals fed a beta-glucan rich diet were significantly more protected against colonisation by *C. difficile* (Table 5). The faecal levels of *C. difficile* were, in animals fed beta-glucan, about 2 log units less than what was detected in animals fed the control diet, one day post challenge ($P < 0.05$). A non-significant reduction of *S. typhimurium* numbers was noted in beta-

giucan treated mice, while a log 3 reduction in mice dosed with *Saccharomyces sp* alone ($P<0.05$). The combination of beta-glucan diet and *Saccharomyces sp* oral administration resulted in a significant reduction ($P<0.05$) in faecal numbers of both pathogens when compared to both the control diet and the *Saccharomyces sp* dosed mice (Table 5). This reduction corresponded to 3 and >4 log units for *C. difficile* and *S. typhimurium*, respectively. This corresponded to a marked improvement in weight loss and other signs of severe infection in animals treated with the combination of beta-glucan and *Saccharomyces sp*. It can be concluded that a beneficial synergistic effect can be achieved by beta-glucan alone and enhanced by the combinations of beta-glucan and *Saccharomyces sp*.

TABLE 5
Reduction of pathogens by oral administration of *Saccharomyces sp* to beta-
glucan fed mice that were challenged with either *C. difficile* or
S. typhimurium

Diet	Reduction (log CFU per g faeces)	
	<i>C. difficile</i>	<i>S. typhimurium</i>
beta-glucan	1.5*	≤ 1
beta-glucan and <i>Saccharomyces sp</i>	3**	$>4^{**}$
<i>Saccharomyces sp</i>	0.8	3*

* Significantly different from beta-glucan free diet ($P<0.05$)
 20 ** Significantly different from beta-glucan-free diet and *Saccharomyces sp* alone.

EXAMPLE 5

This example demonstrates that:

(i) beta-glucanolytic microorganisms are present in the gastrointestinal tract;
 25 (ii) dietary supplementation with beta-glucan stimulates gastrointestinal populations of beta-glucan degrading microorganisms (includes lactic acid-producing microorganisms).

In this study, animals (8 per group) were provided either beta-glucan or control diet (Table 3). After 4 weeks feeding, faecal material was collected, homogenised and diluted in Wilkins-Charlgen broth (Oxoid).

Diluted material was spread on to beta-glucan agar (Table 6) and incubated anaerobically for 75 h. Clear zones developed around colonies formed by beta-glucanolytic microorganisms.

Relative to animals fed the control diet, density of beta-glucanolytic population was up to 100 fold greater in animals fed beta-glucan diet (Fig. 2).

TABLE 6
Composition of beta-glucan agar

Ingredient	Amount (g/L)
Yeast extract	2.5
Trytone	5.0
Peptone	7.5
beta-glucan	10
Cysteine	0.5
NaCl	2.0
K ₂ HPO ₄	2.0
KH ₂ PO ₄	1.0
NaHCO ₃	2.0
MgCl ₂	0.2
CaCl ₂	0.2
CoCl ₂	0.02
MnCl ₂	0.02
FeSO ₄	0.005
Tween 80	2
Hemin	0.005
Vitamin B ₁₂	0.001
Vitamin K	0.0005
Agar	13.0

EXAMPLE 6

This example demonstrates that a range of beta-glucans induce the observed effects. Beta-glucan prepared from a number of *Saccharomyces cerevisiae* strains and of bacterial origin were used. Both crude forms and more purified forms were tested. The crude forms were whole cell sacs that were washed 2-3 times in water and spray dried. The more purified forms were prepared from the water washed preparations that were subsequently alkali washed, then acid washed and then water washed prior to spray drying. When these various beta-glucan forms were incubated with faecal homogenates diluted in laboratory medium, all forms were degraded (Table 7) and were selectively utilised by lactobacilli when tested using lactobacillus pure cultures on glucan agar (Table 6).

15 TABLE 7
Fermentation of beta-glucan preparations by mixed fecal slurries

	Beta-glucan sample				
	1	2	3	4	5
Degradation (%)	50	>60	>60	>70	>50
Cell sac per field:					
0 hour	156	NA	NA	clumped	NA
72 hours	9.2	NA	NA	75	NA
Dry residues (g)	0.3048	0.2630	0.3097	0.3950	NA
Total anaerobes	2.9×10^6	3.1×10^7	1.4×10^7	1.9×10^7	NA

Beta-glucan 1 - water-washed *Saccharomyces* strain A cell sacs

Beta-glucan 2 - alkali- and acid-washed *Saccharomyces* strain B cell sacs (preparation 1)

20 Beta-glucan 3 - alkali- and acid-washed *Saccharomyces* strain B cell sacs (preparation 2)

Beta-glucan 4 - water-washed *Saccharomyces* strain B cell sacs

Beta-glucan 5 - bacterial-origin cell sacs

NA - not analysed

1.3

EXAMPLE 7

This example demonstrates that yeast sac beta-glucan is poorly degraded in the upper regions of the mouse digestive tract and that most of the degradation occurs in the caecum and colon of the animal where the microbial population is greatest. Two groups of three SPF Balb/c mice were fed for 60 hours with a diet containing 40% w/w yeast cell sac beta-glucan. After the mice were sacrificed, the stomach, duodenum, ileum, jejunum, caecum and faeces were sampled. Gastrointestinal contents were squeezed from the gut sections and faecal samples were individually homogenised with 50 mM phosphate buffer and a drop of the homogenate was placed on microscope slides. The degradation of the beta-glucan from mouse intestinal tract samples was examined using phase-contrast microscopy. The beta-glucan granules were degraded by less than 20% in the stomach and small intestine while greater than 70% degradation was noted in the caecum and faecal homogenates (Table 8).

TABLE 8
Degradation of beta-glucan in mouse intestinal tract

	Sample source					
	Stomach	Duodenum	Ilium	Jejunum	Caecum	Faeces
Degradation	< 10%	NO	< 20%	< 20%	> 70%	> 80%

20

NO - Not Observed: Few yeast cell sacs were observed in that area, probably because of the rapid transit time.

USES

The present invention can be applied to all conditions in which microbes are identified or proposed as causative agents of disease in both human and animals and which can be advantageously effected by altering 5 the numbers and/or activities of beneficial microflora of the gastrointestinal tract.

As infective diarrhoea has been shown to be improved by probiotic dosage, the present invention can be used to enhance the effect of the probiotic by itself and to reduce the microbial-induced diarrhoea when orally dosed alone. In addition, the present invention may be used effectively to 10 improve non-infective diarrhoea which has not been shown to be influenced by probiotics alone. It could be effective in reducing the effects of dietary related diarrhoea problems.

Infective diarrhoea refers to all cases of diarrhoea, both acute and 15 chronic, in which the causative agents can be shown to be microbial, including bacterial, viral and protozoan. Such infective diarrhoea can manifest itself in a number of ways e.g. (a) infantile and geriatric diarrhoea; (b) antibiotic associated diarrhoea; (c) traveller's diarrhoea; (d) stressed-induced diarrhoea.

Both prophylactic and therapeutic uses are described in the present specification. The former can relate to prevention when the individual can be exposed to potential problems e.g. (i) investigative gastrointestinal examination when the bowel is decontaminated and can then be recolonised by an undesirable microbial population; (ii) travellers exposed to an altered 20 pathogen load or an alteration of the gut ecosystem because of various factors including diet change, which can predispose the individual to a lower infective dose of a pathogen. Therapeutic uses relate to the treatment of established conditions related to an undesirable balance of the gut microflora or an established pathogen infection.

In summary, glucan preparations singly or together with probiotics and/or prebiotics such as oligosaccharides can be included in foods or other preparations for therapeutic or prophylactic use in the follows situations:

- i. As a general gut microflora stabiliser
- ii. In clinical conditions directly or indirectly related to gastrointestinal microflora e.g. irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), Crohn's disease, diarrhoea and constipation, colon cancer.

sleep disorders, rheumatoid arthritis, chronic fatigue syndrome, hyperactivity, and ulcerative colitis

iii. improved intestinal health

iv. directly influence the metabolism of the microbes such that the beneficial activities are enhanced e.g. elevated levels of antimicrobials or butyrate, and the harmful activities are reduced, e.g. reduced toxin production

v. directly influence numbers of desirable microbes either directly or indirectly in the complex mix of gastrointestinal microbes

vi. directly influence numbers of undesirable microbes either directly or indirectly in the gastrointestinal system.

The present inventors have found that beta-glucans and oligosaccharides are carbohydrates which can be selectively used by beneficial microbes in the gut which in turn can suppress the numbers of the undesirable microbes.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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CLAIMS:

1. A method of enhancing a resident population of lactic acid-producing microorganisms in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganisms and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of the resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided.
2. A method of suppressing an undesired population of microorganisms in a total microorganism population in the gastrointestinal tract of an animal, the method comprising providing to the animal a beta-glucan, compounds derived therefrom, or mixtures thereof, either alone or in combination with other prebiotics and/or with one or more probiotic microorganisms such that upon ingestion the beta-glucan passes through the gastrointestinal tract substantially unutilised until it reaches a site in the gastrointestinal tract where it is utilised by a resident population of microorganism and/or the introduced probiotic microorganisms, if provided, thereby causing an increase in number and/or activity of a resident population of lactic acid-producing microorganisms and the probiotic microorganisms, if provided, and suppressing the growth and/or activity of the undesired population of microorganism.
3. The method according to claim 2 wherein the undesired population of microorganism is an enteric pathogen.
4. The method according to claim 1, 2 or 3 wherein the beta-glucan is a preparation derived from plants, fungi, yeast, or bacteria.
5. The method according to claim 4 wherein the beta-glucan is derived from yeast cell wall preparations or whole yeast cells rich in beta-glucans.
6. The method according to claim 5 wherein the yeast is a *Saccharomyces*.
7. The method according to claim 6 wherein the *Saccharomyces* is *Saccharomyces cerevisiae*.

8. The method according to any one of claims 1 to 7 wherein the beta-glucan is used at a concentration of up to 10% (w/w) when combined with other prebiotics and/or probiotic microorganisms.
9. The method according to claim 8 wherein the beta-glucan is used at a
5 concentration of 1% (w/w).
10. The method according to any one of claims 1 to 9 wherein the resident lactic acid-producing microorganisms are lactobacillus.
11. The method according to claim 10 wherein the lactobacillus is *Lactobacillus fermentum*.
- 10 12. The method according to any one of claims 1 to 11 wherein the probiotic microorganisms are selected from the group consisting of lactic acid-producing microorganisms, bifidobacterium, yeast, and mixtures thereof.
13. The method according to claim 12 wherein the lactic acid-producing
15 microorganisms are lactobacillus.
14. The method according to claim 13 wherein the lactobacillus is *Lactobacillus fermentum*.
15. The method according to claim 12 wherein the yeast is a *Saccharomyces sp.*
- 20 16. The method according to any one of claims 12 to 15 wherein the probiotic microorganism is administered at a concentration of 10^3 to 10^{13} cells per day.

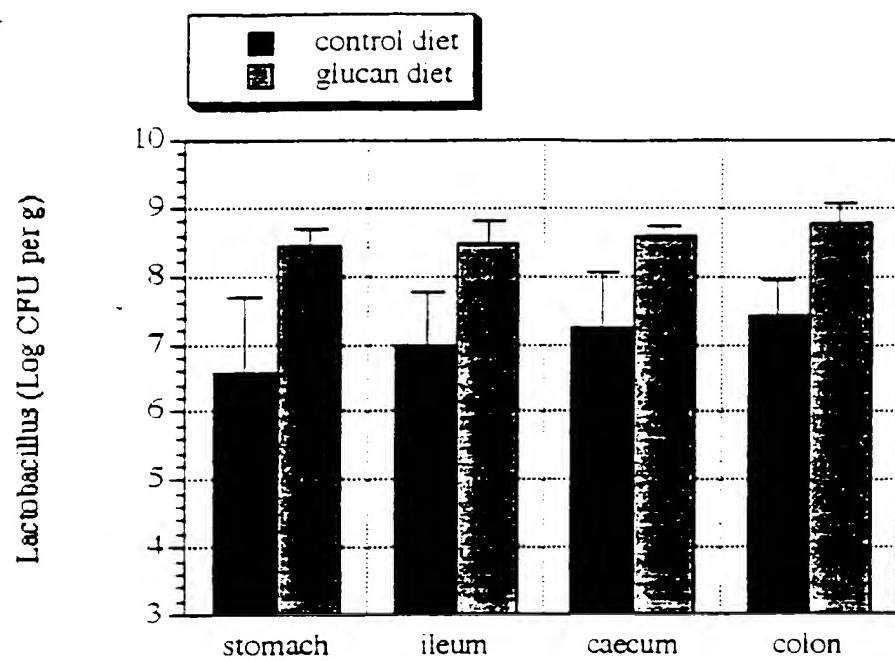
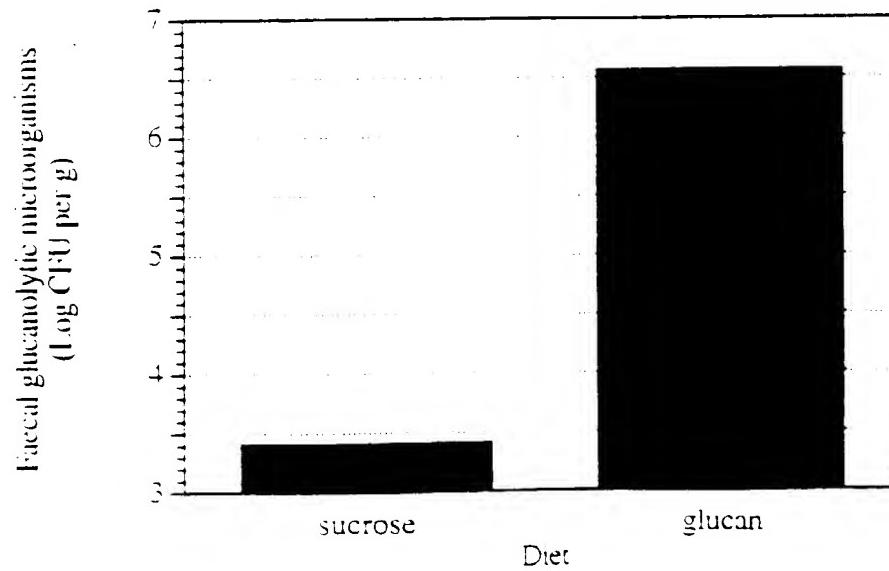


Fig 1



INTERNATIONAL SEARCH REPORT

International Application No

PCT AU 97 00859

A. CLASSIFICATION OF SUBJECT MATTERInt Cl⁰ A61K 031/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 031/715

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 DERWENT DATABASE, glucan, yeast, saccharomyces cerevisiae, bowel, diarrhoea.
 STN INTERNATIONAL KEYWORDS constipation, colon, steen, arthritis, chronic fatigue syndrome, colitis, gastrointestinal, enteric flora, microflora, gut, intestine, lactobacteria

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Patent Abstracts of Japan JP 08157377 A (NIPPON OIL CO LTD) 18 June 1996 - abstract	1-16
X	US 5576015 A (B A DONZIS) 19 November 1996 see whole document	1-16
P,X	AU 61824/96 A (CARLTON AND UNITED BREWERIES LIMITED) 23 January 1997 see whole document	1-16
X	WO 96/26732 A (NEWPHARMA SrL) 6 September 1996 see whole document	1-16

Further documents are listed in the continuation of Box C

See patent family annex

Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"B" earlier document but published on or after the international filing date	"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"C" document which may throw doubts on priority claims or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"D" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"E" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
28 January 1998

Date of mailing of the international search report

10 MAR 1998

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/AU 97/00859

C (Continuation)		
Category *	DOCUMENTS CONSIDERED TO BE RELEVANT	
X	WO 90/04334 A (ALPHA BETA TECHNOLOGY) 3 May 1990 see whole document	1-16
P,X	Front Foods Food Ingredients (1997), 2 (New Technologies for Healthy Foods & Nutraceuticals), pages 53-70 Manssur Yalpani, "Nutraceuticals - A Materials-Based Perspective" see in particular pages 63-65	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/AU 97/00859

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
AU	61824/96	WO	97/0123				
WO	90/04334	CA	900428	AU	44924/89	DE	940707
		EP	910814	JP	921022	NZ	970526
		US	901009	ZA	911030		

END OF ANNEX